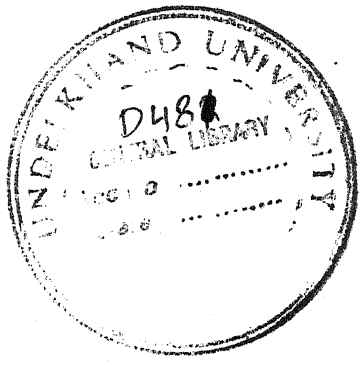
ESTIMATION OF SECRETORY IMMUNOGLOBULINS IN CERVICAL MUCUS

THESIS

FOR

MASTER OF SURGERY

(OBSTETRICS & GYNAECOLOGY)



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C E R T I F I C A T E

This is to certify that the work entitled "ESTIMATION OF SECRETORY IMMUNOGLOBULINS IN CERVICAL MUCUS", which is being submitted as a thesis for M.S.(Obstetrics and Gynaecology) by DR. NAZMA SABIHA has been carried out in the Department of Obstetrics and Gynaecology, M.L.B. Medical College, Jhansi.

She has put in the necessary stay in the department as per University regulations.

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The techniques embodied in the thesis were undertaken by the candidate herself and observations recorded have been periodically checked and verified by me.

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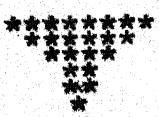
In the last I am emotionally overwhelmed to dedicate the present work to the memory of my respected father whose impeceable nature, irrevocable decision have left an iddefinable impression in my heart and whose memory shall always enlighten my future.

Nazma

(NAZMA SABIHA)

I N D E X



INTRODUCTION

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The human female genital tract appears to be an immunologically reactive tissue. Antibodies to a variety of microbial antigens have been observed in the secretions of the genital tract.

In recent years the analysis of the protein components of the cervical mucus besides the mucoid substace itself has become more and more interesting particularly with respect to fertility problems.

The human endocervix is lined with secretory columnar epithelium which produces mucus resembling the unboiled white of egg. Cervix secretes about 20-60 mg of mucus/day which increases to 700 mg/day during mid menstrual cycle of a women's reproductive life. The menstrual cycle has a profound effect on the nature and quality of the cervical secretions. Cervical mucus forms a network of macro-molecules of mucin made up of glyoprotein micromolecules arranged in long chains. This mucus network is filled with cervical plasma. The biochemical composition of the mucus comprises of water 85-90% and mucoids and mucins (25% amino acid chains 75% galactose) sialic acid, albumin, globulin, lipo-protein, immunoglobulin, lactoferrim and certain enzymes like alkaline phosphate, glucuronidase and

diastase, cholesterol, sodium chloride potassium and prostagladin. Under oestrogenic stimulation, the glyoprotein network is arranged parallel to each other, thus helping in speram penetration. During ovulation at mid cycle the cervical mucus becomes copius, more elastic less viscous and less cellular while following ovulation during the progestogenic phase of the menstrual cycle, the glyoprotein network becomes more dense with interlacing bridges precluding sperm penetration.

The immunoglobulins in the cervical mucus originates by passive diffusion from the serum (Van Kaulla et al 1957) from the follicular fluid via the fallopian tube (Harwe et al 1965) or be produced locally in the cervix in response to a local antigenic stimulus from a gonococcal trichomonal or monilial genital tract infections (Chipperfeild et al and Evans 1972). Hutcheson et al (1974) have incriminated local antibody in the aetiology of infertility.

The detection of secretory peice and lactoferrin in endocervical epithelium by immunofluorescene (Masson Hereman and Ferin 1968, Hulka and Omran 1969, Tourville e et al 1970) and the location of IgA and IgG containing plasma cells in the underlying stroma by the same method further add support to the local production of antibody

by the cervical mucus. This immunoglobulin production in the endocervical sub-mucosa might be considered as a mechanism required to prevent pathogenic invasion of the uterus and tubes.

Patients with abnormal cervical cytology had significantly raised immunoglobulin concentrations particularly IgA (Coughlan and Skinnar 1977), hence a prior status of immunoglobulin if known in normal women would help to diagnose earlier dysplastic changes in the cervix and could thus act as a prophylactic procedure in diagnosing the benign conditions which would progress into malignancy.

The relative and absolute concentrations of the immunoglobulin IgA and IgG in cervical mucus appear to vary in the different phases of menstrual cycle. Eslein (1970) studied the effect of progestogen on the protein of cervical mucus. At midcycle only albumin was present in most of the cases. Immunoglobulin and transferrin were normally not identifiable, shortly after ovulation transferrin and immunoglobulin reappeared in the mucus with immunoglobulins becoming quite prominent and diminishing towards the end of the luteal phase. Thus the progesterone influence on cervical mucus was manifest as an increase in the protein concentration. This

increase of immunoglobulin concentration may serve to protect the early conceptus from potentially pathogenic infections agents.

Maternofoetal immunologic relationship is a unique phenomenon in the biologic world. Foetus is considered as homograft transplant of living tissue between genetically dissimilar members of the same species. The success of conception as a graft has been attributed to an integrated multifactorial system which maintain a dynamic equilibrium throughout pregnancy. The foetus and placenta containing paternally determined antigens foreign to the mother in which they are developing, seem to escape the immunoglobin distruction and survive for 40 weeks with no evidence of rejection at birth.

The trophoblast which forms an operational interface between mother and conceptus passes into maternal circulation during normal pregnancy acting as a tissue antigen. These antigens evoke the production of immunoglobulins IgM and IgG of which IgG crosses the placental barrier. The placental barrier prevents the massive transfer of lymphocytes and is responsible for the protection of the foetus from maternal immunologic attack.

Immunoglobulin (IgG) mainly develops in secondary response to antigen and it is the only antibody which is selectively transferred across the placenta, thereby giving a measure of protection to the newborn infant.

Immunoglobulin IgA does not cross the placenta and is synthesized by the foetus in small amounts. Immunoglobulin M (IgM) antibodies are first to be produced in primary responses and replaced subsequently by IgG antibodies. It is a macroglobulin and hence fails to cross the placenta. IgM present at birth is predominantly of foetal origin and it provides protection to the foetus from many pathogens.

In pregnancy the mean serum levels of IgG and IgM are significantly raised which suggests that immunization to foetal antigen stimulated the maturation of maternal immune system which is necessary to prevent the rejection of foetal allograft the value of IgG are highest in the first trimester and there is a gradual decrease in the second and third trimester. This fall in attributed to the passive transfer of IgG to foetus in its intrauterine life.

Thus the melieu of cervical secretion should be reflected by a change in the serum concentrations of the immunoglobulin IgG levels. Thus the changing IgG

levels in the cervical secretion and the deviations from normal might be suggestive of some mishap occuring with the mother or the foetus in utero and can be promptly managed.

Besides the cervix acting as a locally antigenic tissue also produces certain secretory immunoglobulin (IgA) which have their own protective influences on the female genital organ.

Pelvic inflammatory disease a common infection in women is having an increasing prevalence since 1960. The natural barrier to pelvic infection is the cervix where a downward flow of the mucus and the action of cilia are augumented by the production of a lysozyme. Aided by the presence of cervically secreted IgA the lyozyme hydrolyes the peptiglycan links of microorganisms allowing osmotic destruction. This cervical barrier may be compromised after miscarriage, abortion, childbirth, cervical surgery and in the presence of a intra uterine contraceptive device. The immunoglobulins thus serving to protect the female genital organ from the dreaded pelvic inflammatory disease and the subsequent effect of the disease on the pregnancy outcome of a normal female.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Mens truation is a function peculiar to women. It is defined as the periodic and cyclical shedding of the progestational endometrium accompanied by loss of blood. It takes place approximately at 28 days interval between the menarche and the menopause. There is an intimate relationship between the hypothalenus, the pitutary gland the ovaries and the uterus. Each of which influences the functions of the other.

It is not known what triggers the hypothalanus to release GnRH in years prior to puberty, but it is thought that repeated small stimuli from GnRH induces the gonadotroph cells of the anterior pitutary to synthesize and to release small amounts of gonadotrophin into the circulation until eventually sufficient amounts are released to stimulate ovarian activity. Initially FSH is released with only a small amounts of LH.

Above a critical circulating concentration FSH stimulates a few esensitized follicles in the ovary to mature. FSH also induces the theca cells which sorround these follicles to secrete increasing amounts of oestrogen. The rising concentration of oestrogen feeds back to the pitutory and the hypothalamus in a negative way reducing the amount of FSH oestrogen levels continue to rise to reach a peak.

The uterus is influenced by these rising levels of oestrogen causing proliferation of the endometrium stimulating the growth of the glands and compacting the stroma. It increases the vascularity of the organ and causes the development of the mymetrium. The midcycle surge of oestradiol increases the sensitivity of the pitutary gandotrophs and induces a surge release of GnRH. This is the positive feed back. The GnRH surge induces a small FSH and a large LH surge, ovuluation occurs about 30 hours after the LH surge.

Following ovulation in the secretory phase of the menstrual cycle. The theca granulosa are luteinized and begin to secrete progesterone and a curpos luteum is formed by the 5th day of ovulation the corpus luteum is fully functioning.

The circulating progesterone added to the circulating oestrogen exerts a negative feed back on the hypothalamus and the pitutary causing a fall in levels of FSH and LH. Unless the released ovum is fertilized and implants within 7 days of ovulation, the corpus luteum begins to degenerate with a rapid feduction in oestrogen and progesterne. This is insufficient to support the endometrium and approximately 14±2 days after ovulation the endometerium breaks down and its blood vessels rupture causing bleeding.

THE CERVICAL CYCLE

The cervix uteri is influenced by the waxing and waning secretions of the female sex hormones. In the folicular phase the cells lining the clefts of the cervical canal/proliferate an actively secrete a thin watery mucus. This is most marked at the time of ovulation when a cascade of mucus can be seen. During the follicular phase the cervical mucus absorbs water and salts and when allowed to dry deposits crystals of sodium and potassium chloride. The ovulation cascade is related to a low content of proteins in the cervical mucus.

During the luteal phase the cervical glands become more branched and their secretion changes its physical and chemical properties. The mucus becomes more viscous and forms a more secure cervical plug. These changes are related to an increase in the amount of proteins in the mucus and to the presence of phospholipids under the influence of progesterone. The electrolyte content of the mucus is also reduced. During mensturation the cervical glands collapse and may show slight desquamation with loss of blood.

CERVICAL SECRETIONS

(C.S. Dawn, 1985. Text book of Gynaecology and Contraception, 17-18).

Cervical secretion is an alkaline mucus like the unboiled white of an egg. Cervix secretes 20-60 mg of mucus/day which increases to 700 mg/day during the menstrual cycle of a women's reproductive life. The menstrual cycle changes the cervical secretions.

BIOCHEMICAL COMPOSITION

Water 85-98% and mucoids and mucins, 25% amino acid chains 75%, glactose, sialic acid, albumin, globulin lipoprotein immunoglobulins, lactoferrin, enzymes like alkaline phosphatase, glucuronidase, diastase, glucose, cholesterol, sodium chloride, potassium and prostaglandins. The secretory immunoglobulins are considered as the first line of defense.

IMMUNOGLOBULINS

The antibody activity of serum andother body secretions is associated with a hetrogenous group of proteins collectively known as immunoglobulin. These proteins are also known as gama globulins because of the relative electrophoretic mobility. Many antibodies migrate more rapidly than gamaglobulins and some molecules unrelated to antibodies may also migrate with the electrophoretic mobility of gamaglobulins. For these reasons the term "immunoglobulins" and symbol Ig has been suggested to designate the family

of molecules with antibody activity (Committee of nomenclature of human immunoglobulin Bull. W.H.O. 1964, Fahey, 1965).

Gross et al (1959) described three main types of immunoglobulins with antibody activity which are immunoglobulin G (IgG), immunoglobulin M (IgM) and immunoglobulin A (IgA). Recently two more proteins with immunochemical characteristics related to these immunoglobulins like immunoglobulin D (IgD), and immunoglobulin E (IgE) has been detected. Despite the tremendous haterogenicity all the immunoglobulins shown structural similarity.

Immunoglobulin molecules are composed of two kinds of polypeptide chains. Each molecule consists of large identical polypeptide chains referred to as heavy chains and two identical smaller ones referred to as light chains. These polypeptide chains are held together by disulfide bonds and by non covalent bonds which are primarily hydrophobic. The heavy and light polypeptide chains are synthesized on separate ribosomes assembled in the chains and secreted as an intact molecule. Five immunoglobulin classes (IgG, IgA, IgM, IgD and IgE) are recognised on the basis of structural differences of their heavy chains.

IMMUNOGLOBULIN G (IgG)

Immunoglobulin G (IgG) is the major immunoglobulin and constitutes about 3/4 of the total gamaglobulin. The serum concentration varies from 800-1600 mg/dl in adults but intravascular pool accounts for less than half of total body immunoglobulin. About 55% is widely distributed within the extra vascular spaces. IgG molecules have half life of about 21 days and are the longest lived immunoglobulins. The total body content is in excess of 1 mg/kg body weight. On the basis of antigenic determinants within the heavy chains of IgG four isotypic sub classes of IgG molecules have been identified in the normal serum. These are IgG_1 66%, IgG_2 23%, IgG_3 7% and IgG_4 4%. It is the only globulin which is selectively transferred across the placenta giving major pretection to new born infant (Fahey, 1965).

SECRETARY IMMUNOGLOBULINS

IgA is the predominant immunoglobulin in external secretions of the respiratory tree, GIT and the genito urinary systems, in tears, saliva and colostrum. The IgA producing plasma cells are the predominant plasma cells in the sub mucosa. Secretary IgA is composed up to two IgA molecules bound to a secretory piece by disulfide bonds. The secretory piece is a polypeptide

chain with a molecular weight of 70000 daltons that is synthesized by epithelial cells. The dimeric IgA is held together by a single J. chain which is also synthesized by sub mucosal plasma cells. Once the dimeric IgA chains leaves the plasma cells it enters the epithelial cells and becomes covalently bound with the secretory piece. It is then secreted in the lumen. Secretory IgA can have antibody activity to bacterial and viral antigens and toxins. They bind microorganisms and prevent their attachment to epithelial cells and administration of antigens have resulted in an enhanced production of secretory IgA in the respective organs.

IMMUNOGLOBULIN M

Immunoglobulin M is the protein with molecular weight 850000 daltons. IgM is also known as macroglogulin due to its high molecular weight. The rate of synthesis of IgM is only i/20 of IgG where as their fractional catabolic rate is 2-3 times that of IgG (half life 5 days) and this accounts for low serum IgM levels (80-300 mm/dl). Little if any IgM crosses the placental barrier and the IgM present at birth is predominantly of foetal origin.

Tiselius (1937) reported fall in gammaglobulin protein in the IIIrd trimester of pregnancy and he

corelated this fall with transfer of immunity from mother to the foetus.

Brown in 1954 studied for the first time the stagewise alteration of immune globulin in pregnancy and suggested that gammafraction of immunoglobulin is unaltered in the first trimester. Thereafter it decreases steadily in late pregnancy.

The mean IgG levels in pregnancy found by Gusdon (1969) was somewhat higher than recorded by others. Serum IgG concentration throughout later half of pregnancy differ in that they are consistantly higher by 200 mg% at each week of gestation in comparison to others. Maternal and foetal IgG were equalled at 33 weeks and after that mean foetal IgG were slightly higher than maternal values.

Best et al (1969) reported greatest fall in serum IgG and IgA levels during pregnancy and this fall is linear throughout pregnancy.

Maroulis et al (1971) observed that concentration of serum IgG diminishes with each successive trimester, whereas concentration of other two immunoglobulin IgM and IgA were either unchanged or had no consistent trend. The lowering of serum IgA concentration was statistically significant. They found statistically

significant difference of IgG concentration between second andthird trimester and control group.

Ganguli et al (1980) reported a decrease in IgG levels during pregnancy upto parturition IgG and IgM levels were higher in the first trimester than those of controls. The raised IgG and IgM levels dropped to normal during terminal stage of pregnancy with further fall in IgG and IgM levels at the time of parturition. No change was observed in IgA levels during pregnancy.

In 1941 Kerr W.R. and Robertson,M. first suggested the production of local antibodies by the vagina who observed the presence of agglutinins to trichomonally affected foetus of heifers.

In 1943 Kerr and Robertson again observed the same findings in infected heifers they also showed that these antibodies were probably responsible for the disappearance of the organism from the vagina after oestrus.

Pierce (1946) has found that agglutinating antibody to trichomonas foetus appears in the vaginal mucus of infected cows earlier at higher titres than in the serum.

Kerr and Robertson (1953) demonstrated the independance of local vaginal and serum antibodies in trichomoniasis of cattle confirming their earlier

observation which further strengthened the view that local production of antibodies occur in the female genital tract.

Huges (1937) gathered the evidence of local vaginal antibody production against foetus affected by vibrio in cattle.

Batty and Warral (1955) have observed the evidence of local production of antibodies against diptheria toxoid and tetanus toxoid in vaginal and uterine walls.

Kerr (1955) has demonstrated that local antibodies are foundiin vaginal mucus when cattle are infected with bordetella abortus.

Ven Kaulla et al (1957) have reported from their studies with radioisotape labelled (I^{13}) albumin that exogenously administered (I/V route in their case) substances were rapidly excreted by the human cervical glands and are concentrated in the cervical mucus. They have also observed that patients in the progestational phase of the menstrual cycle. Seemed to concentrate radioiodine in the cervical mucus more rapidly and to a higher levels than those in the early phase of the menstrual cycle.

Lindhl et al (1956) reported the appearance of antisperm agglutinim in the cervical secretion of

human females shortly after ovulation and in the second trimester of pregnancy.

In 1958 Geslonitiz et al have demonstrated the presence of anti A and anti B agglutinins in the cervical secretions of normal women.

Teponga (1959) has confirmed the findings of earlier workers that cattles infected with vibro foetus also showed local antibody production to these organism in the vaginal mucus.

Strauss (1961) observed the occurence of antibodies to typhoid bacillus following local inoculation in human vaginal mucus. She further noticed better response to vaginal inoculation for production of local antibodies as compared to production of serum antibodies following parenteral immunization in human females.

Solish et al (1961) studied the random samples of human cervical mucus (82) and observed the presence of isohaemagglutinins observation on multiple samples obtained from 41 women failed to suggest any correlation between antibody titre and phase of menstrual cycle.

Moghissi et al (1962) again confirmed the presence of gammaglobulins in human cervical mucus. They hypothesized a role of these gammaglobulins in fertility.

Anzoi et al (1963) also reported the presence of gammaglobulins in human cervical mucus.

Chodirkar and Tomasi (1963) studied three types of gammaglobulins (γ^2, γ^{1A} and γ^{1m}) in human serum and various body fluids including vaginal fluid. They found that in vaginal fluid the rates of γ^2/γ^{1A} did not differ significantly from that of the serum.

Firemans et al (1963) reported that in cervico vaginal secretion IgA:IgG ratio is higher than that of serum and that antibody present in the secretion belongs to the secretory IgA class.

Schumacher et al (1965) studied serum proteins in human cervical mucus using immoelectrophortic methods. They analysed cervical mucus samples during various phases of menstrual cycle in normal women. They could not find any significant difference in gammaglobulin levels during these phases. They also noticed significantly higher levels of gammaglobulin in cervical mucus of patients suffering ffrom cervical erosion and chronic cervicitis.

Moghissi and Nenhaus (1966) did not find any remarkable change in the cervical mucus immunoglobulins throughout the menstrual cycle in four fertile normal healthy women.

Bell and Wolf (1967) demonstrated the invitro production of antibodies against diptheria toxoid by rabbit vagina following direct exposure of rabbit vagina to the antigen in vivo. They could not find any invitro production of antibodies by rabbits uterus during the same study.

Parish et al (1967) studied the human cervical mucus during the non menstrual interval in selected women, cervical mucus extracts contained gammaglobulins and several other types of serum proteins. These globulins included agglutinating antibodies to the A and B blood group antigens and immune type haemolytic anti A and anti B antibodies to E. coli and condida albicans. In eleven women certain antibodies were present in cervical mucus but not in serum pointing towards local antibody production.

Elstein and Pollard (1968) demonstrated the presence of immunoglobulins in progestational cervical mucus while noting down the variation of albumin level in cervical mucus in various phases of menstrual cycle.

Parish and Ward (1968) studied cervical mucus of three infertile women where other causes of infertility were excluded. They found that one woman had an IgG cytoxic antibody in her serum and cervical mucus which

caused complement mediated disruption of spermatozoal head, the second woman had antispermatozoal cytotoxic antibody IgG of immobilizing type. Besides these IgG three, harmless immunoglobulins (IgG and IgA) class were also found in the three women.

Masson et al (1969) analysed biochemical composition of pooled human cervical mucus and reported the concentration of various immunoglobulin i.e. IgA 0.090 mg/ml. IgG 0.45 mg/ml, IgM 0.035 mg/ml. The IgA and IgG ratio was thus 1:5 which approximately equal to that of serum.

Hulka and Omran (1969) studied cervical mucus from 7 women with proven fertility. IgA was found to be present in concentration of 2.8-90 mg/100 ml and IgG was present in concentration of 9-4.56 mg/100 ml. IgA/IgG ratio tended to increase as the cervical mucus volume increased at mid cycle.

Elstein (1970) studied the effect of progesterone on proteins of cervical mucus. At mid cycle only albumin was present in most of the cases.

Immunoglobulin and transferrin were normally not identifiable. Shortly after ovulation transferrin and immunoglobulin reappeared in the mucus with immunoglobulins becoming quite prominent and diminishing towards

the end of luteal phase. The progesterone influence on cervical mucus was manifest as an increase in protein concentration and appearance of prominent banas of transferin and immunoglobulin.

Tourville et al (1970) made immunofluroscent studies on various tissues of human female reproductive tract including uterine tube. Endometrial, cervical and vaginal biospy material. They demonstrated the presence of, G and A and M immunoglobulins in these tissues. G and A were the prodominant immunoglobulin. Both G and A increased during the secretory phase. Moreover secretory protein was also present in these tissues thus confirming the fact that local secretion of IgA occurs in these tissues.

Waldman et al (1971) detected immunoglobulin levels in cervicovaginal secretions from 131 normal women. IgA was found in 117 with a mean of 0.22 mg/ml. IgG in 124 females with a mean of 0.12 mg/ml and IgM in 41 with a mean of 0.01 mg/ml. In 74 samples IgA was the predominant immunoglobulin class in 48 IgG was predominant and in 9 patients the two were equal. The percentage of the total immunoglobulin that was IgA averaged 69% of all the samples. In older age groups the percentage of total immunoglobulins that was IgA

decreased and IgG increased. There was significantly greater percentage of IgG in the cervico vaginal secretion of women over 50 as compared to women below 30 ($P < 0.05$). In a variety of conditions such as pregnancy, sterility vaginitis etc. the IgA:IgG ratio in the cervicovaginal secretions remained unchanged. The relation with the phases of menstrual cycle also caused no significant changes in the immunoglobulin composition of the cervico vaginal secretion.

Chipperfield and Evans (1972) studied the formation of immunoglobulin in the lamina propria of the endocervix in relation to specific acute local infection. Plasma cells containing IgA, IgG, IgM were identified immunohistochemically by the direct fluroscent antibody method in specimens obtained by needle biopsy. Infection by Neisseria gomorrhoeae trichomonas vaginitis a condida albicans was associated with an increase in the number of fluroescing plasma cells in all three classes, but predominantly IgA; plasma cells of IgM class were more prominent in trichomoniasis than in the other two infection. There was no apparent relationship with menstrual cycle, oral contraceptives or cervical erosion. They suggested that immunoglobulin production in the endocervical submucosa might be

considered as a mechanism required to prevent pathogenic invasion of the uterus and tubes.

Waldman et al (1972) immunized 10 women intravaginally with candida albicons vaccine antibody response in cervicovaginal secretions was predominantly IgA type. There was no coincident rise in serum IgA type levels indicating local production of IgA in cervicovaginal secretion. They postulated a role of these antibodies in opsonization of candida albicons or complement mediated distruction of these organisms. Ogra and Ogra (1973) studied antibody response to poliovious type I in serum, the nasopharynz and the secretions of the genital tract in human volunteer after intravaginal and intrauterine nasopharnygeal or intermuscular immunization with inactivated poliovaccines. Intravaginal and intrauterine immunization consistently resulted in the appearance of secretory antibody to poliovirus in the genital tract. The vaginal response was predominantly of A immunoglobulin while the response in the uterus was essentially limited to G immunoglobulin. Intramuscular immunization resulted in a delayed appearance of G response in the genital tract which could be corelated with the highest G antibody titres in the serum. No genital A response

was observed, however after such immunization these observations provided evidence for local synthesis of poliovirus antibody in the genital tract in the view of these workers.

B.M. Coughlan and Skinner (1977) studied the immunoglobulin concentrations in cervical mucus in patients with normal and abnormal cervical cytology. IgG and IgA were present in every mucus samples while IgM was only occasionally seen in some. There was an increase in the levels of IgG and IgA towards the last week of the menstrual cycle, None so far IgA. These increasing levels may serve to protect the early conceptos from potentially pathogenic infectious agents similarly patients with abnormal cervical cytology showed increased IgG and mere strikingly IgA concentration but there was no corelation between the two at any stage of the menstrual cycle. Whereas in patients with normal cervical cytology, the IgG and IgA concentrations corelated throughout the menstrual cycle. The increase in IgA concentrations in patients with abnormal cervical cytology may represent a local response to abnormal epithelial cells or to a prolonged antigenic stimulus from an exogenous or endogenous infection agent.

Amino et al (1978) studied the changes of serum antithyroid antibodies during and after pregnancy in autoimmune thyroid disease. They found a decrease in the levels of both antithyroid haeagglution antibodies (IGHA) and antithyroid microsomal haemagglutination antibodies (MCHA) during pregnancy and a increase in the titra of these antibodies was seen after delivery. Similar transient increases in antibodies were observed after spontaneous and therapeutic abortion - These changes are attributed to the fact that maternal immunological inertness has been postulated as one mechanism for protecting the foetal allograft, as a consequence of which the levels of serum concentration of IgG, IgA and IgM should be decrease in normal pregnancy. Haemodilution occuring during pregnancy might also contribute to a the reduction in the levels. Hormonal changes associated with gestation such as increased free serum cortisol and choriomic goadotorpin have an effect on the hemostatic immune regulation preventing rejection of the foetal allograft.

Gulbir Bhatia et al (1981) studied the changes in the levels of secretory immunoglobulin in cervical mucus in normal patients with respect to the different phases of menstrual cycle, the three trimesters of

pregnancy and menopause and in women suffering from cervical pathology. As the secretory immunoglobulin are thought to represent a first line of defence a known ledge of the changes occuring consequent to infections might serve as a predictor for the graver pathologies of the cervix including malignancies. There was a decrease in the levels of IgA and IgG in mid cycle and maximum concentration was seen towards the end of the cycle. The mean values of IgG being more than IgA.

The levels of IgG and IgA were also lower in the three trimesters of pregnancy when compared to the premenstrual levels, the ratio of IgG:IgA was found to be decreased in 2nd trimester as compared to Ist and IInd.

In pathological condition of the cervix both immunoglobulin were raised as compared to normal women and especially IgG was significantly increased. The increasing levels premenstrually are attributed to hormonal changes on the cervical mucus. Estrogen are known to cause a decrease in the concentration of immunoglobulin, whereas progesterone increases the concentration. The levels of immunoglobulins are found lower in pregnancy, due to immunosuppression occuring during pregnancy to save the foetus being a allograft in the maternal tissue.

MATERIAL AND METHODS

MATERIAL AND METHODS

The present study was conducted on 55 (women in the department of Obstetrics and Gynaecology, M.L.B. Medical College, Jhansi over a period of one year.

SELECTION OF CASES

For the purpose of this study patients were selected from the out patient department and the indoor patients of the wards and were grouped into the following categories :-

A. Women in different phases of menstrual cycle :
   (a) Pre menstrual
   (b) Mid cycle
   (c) Post menstrual

B. Women in three trimesters of pregnancy.

C. Women suffering from pathological conditions of the cervics :
   (a) Chronic cervicitis -
      i) Endocervicitis
      ii) Cervical erosion
   (b) Cervical dyplasis

Clinical history of patients regarding their age, socio-economic status, their menstrual history, obstetrical history, dietary history was taken and their general examination was done.

In the pregnant patients the fundal height was assessed and the period of gestation was determined by :

(a) P/V examination was done in the patients of Ist trimester pregnancy to assess the size of the uterus and for the confirmation of pregnancy.

(b) P/A examination was done in the 2nd and 3rd trimesters of pregnancy.

(c) P/S examination was done in patients selected as cases of having a cervical pathology on the basis of their history of low backache, vaginal discharge and general ill health.

COLLECTION OF CERVICAL MUCUS

The patients were laid down in the dorsal position and after clearing the vagina with an anticeptic solution, a posterior vaginal wall speenlum was applied the anterior lip of the non-pregnant cervix was held by a volsellum and that of the pregnant cervix with a sponge holding forcep. Next 0.5 mg of cervical mucus was collected with the help of a tuberculin syringe. Samples contaminates with blood were rejected. The collected samples were mixed with a known volume of normal saline depending upon the consistency of cervical mucus and stores at -20°C.

EXAMINATION OF IMMUNOGLOBULINS IN CERVICAL MUCUS (IgA and IgG)

For quantitative estimation of serum immunoglobulins Single Radial immunodiffusion technique as described by Mancini et al (1965) was used :

Principle

The principle of this technique is that a antigen diffuses radially from the point of application into an antibody containing gel and a circular precipitin ring forms at the zone of equivalence keeping antibody concentration and gel thickness constant, the area covered by the precipitin ring is proportional to the concentration of the antigen.

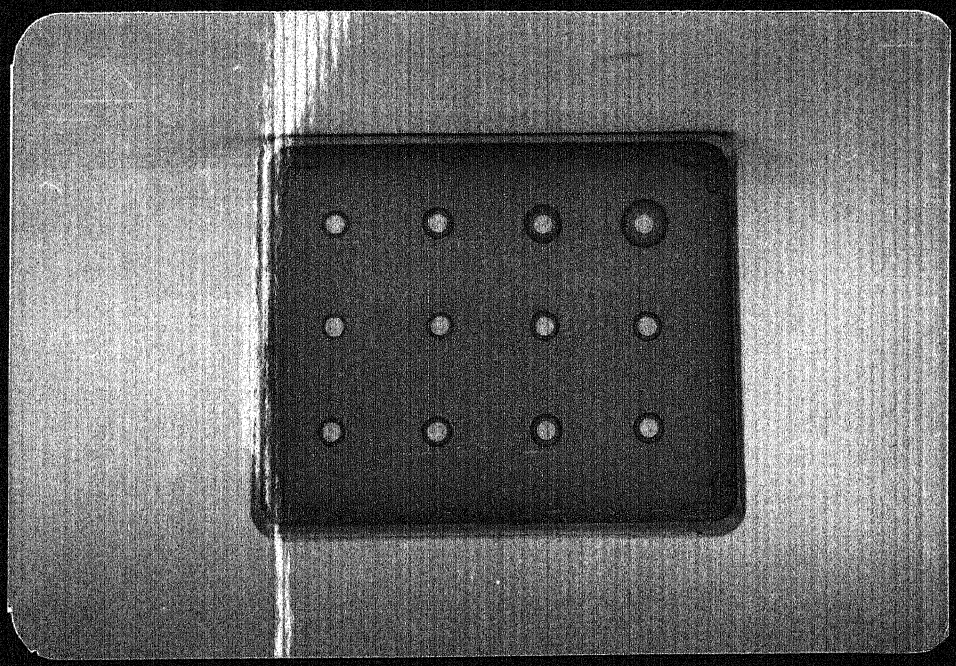
IgA and IgG levels in cervical mucus were measured by the above technique using commercially available immunodiffusion plates.

Reference serum provided by the manufacturer having known quantities of IgG/IgA was fixed in three wells in the concentration of 100%, 50% and 25%.

Each well was filled with 5 microlitres of test material, after filling the wells, the plates were kept aside for 10 minutes to dry and then incubated at room temperature in humid atmosphere for 24 hours. Diameter of each precipitin ring was measured after 24 hours of

incubation to the nearest 0.1 mm. Diameter square of the reference was used to plot a standard graph.

Against this standard graph, value of unknown samples corresponding to their diameter squares were read. These readings were taken as levels of IgG/IgA in cervical mucus samples after correction of dilution factor (1:1).



PRECIPITIN RINGS - SHOWING CONCENTRATION OF IG's IN CERVICAL MUCUS

OBSERVATIONS

OBSERVATIONS

The present study was conducted in the Departmentss of Obstetrics and Gynaecology and Pathology, M.L.B. Medical College, Jhansi over a period of one year from May 1990 to April, 1991. There were several groups of subjects in the study as indicated below in table No.-I.

Table - I

Sl. No.	Type of subject	No. of subjects
A1	Premenstrual (normal) women	7
A2	Mid cycle (normal) women	7
A3	Post menstrual (normal) women	7
A	Normal controls	21
B1	Women in first trimester of pregnancy	7
B2	Women in second trimester of pregnancy	7
B3	Women in third trimester of pregnancy	6
B	Pregnant females	20
C1	Patients with cervical erosion or endocervicitis	9
C2	Patients with cervical dysplasia	5
C	Patients with cervical pathology	14

Average age of the patients

The average age of females in the control group was 21.2 years ranging from 18 to 25 years.

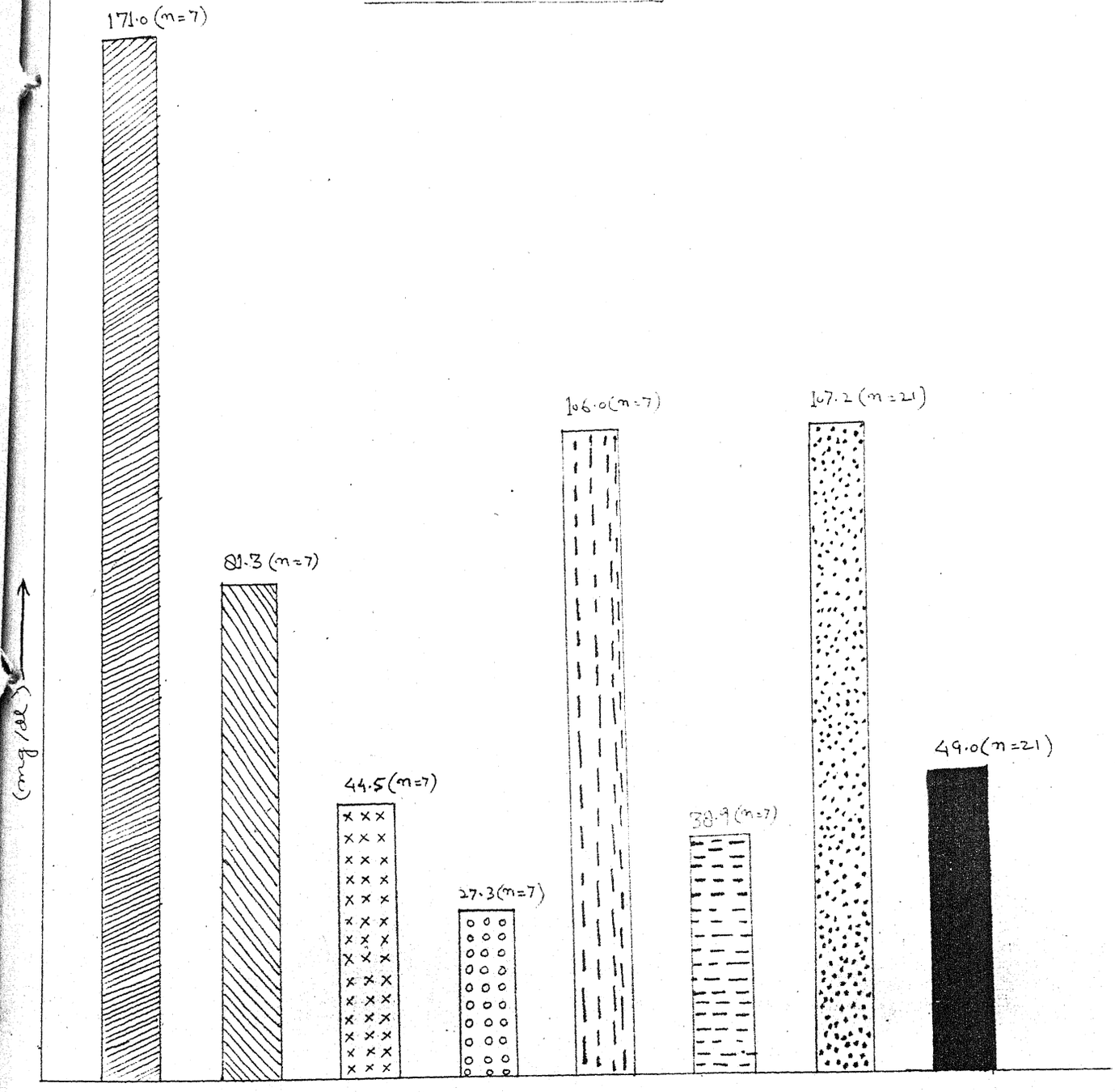
While those in pregnant group averaged 21.9 years (range 18-29 years).

Table - II(a)

Immunoglobulin (Ig) levels in cervical mucus of women during different phases of menstrual cycle

Sl.No. of subj-ects	Phase of menstur-al cycle	IgG level (mg/dl)	IgA level (mg/dl)	IgG/ IgA ratio	Significance of differe-nce
35	Premenstrual	112.2	41.5	2.7	Between group
36	- do -	237.5	123.5	1.9	A1 & group A2
37	- do -	207.0	78.5	2.6	for IgG levels
38	- do -	171.0	84.0	2.0	highly signifi-
39	- do -	121.0	57.5	2.0	cant. P $\angle$ 0.001.
40	- do -	147.5	75.0	2.0	For IgA levels
41	- do -	201.5	108.0	1.9	highly signifi-
					cant P $\angle$ 0.01.
Group A1 (n=7) mean $\pm$ S.D.		171.0$\pm$46.9	81.1$\pm$28.0	2.1	
42	Mid cycle	31.5	0		Between group
43	- do -	27.5	0		A2 and A3 for
44	- do -	67.5	59.0		IgG highly
45	- do -	0	0		significant
46	- do -	84.0	56.5		P $\angle$ 0.001 for
47	- do -	78.0	48.5		IgA not sign-
48	- do -	82.0	27.0		ificant P $\nearrow$ 0.1
Group A2 (n=7) Mean $\pm$ S.D.		44.5$\pm$34.3	27.3$\pm$27.5	1.6	
49	Post menstrual	89.0	34.5	2.6	Between group
50	- do -	108.5	48.5	2.3	A1 and A3 for
51	- do -	118.5	66.0	1.8	IgG and IgA both
52	- do -	88.0	15.0	5.9	highly signifi-
53	- do -	94.0	37.5	2.5	cant P $\angle$ 0.01
54	- do -	113.5	21.0	5.4	
55	- do -	131.0	50.0	2.6	
Group A3 (n=7) mean $\pm$ S.D.		106.0$\pm$15.3	38.9$\pm$17.6	2.7	
Group A (n=21) (control) Mean $\pm$ S.D.		107.2$\pm$59.4	49.0$\pm$35.3	2.2	

Diag (1) – Immunoglobulin levels in normal controls.
(in cervical mucus)



Immunoglobulin levels in cervical mucus of normal control women

As shown in the table II mean value of IgG±SD (mg/dl) in cervical mucus of women in premenstrual phase was 171.0±46.9 while in mid cycle and post menstrual phase these values were 44.5±34.3 and 106.3±15.3, respectively. IgG level in mid cycle was significantly lower than either premenstrual level or postmenstrual level (P value < 0.01). When Ig level was compared between premenstrual and postmenstrual phases, it was significantly higher in premenstrual phase than in post menstrual phase (P value < 0.01).

Mean IgA values ± SD (mg/dl) in premenstrual mid cycle, and post menstrual periods were 81.1±28.0 27.3±27.5 and 38.9±17.6 respectively. Premenstrual IgA level was significantly higher than those during mid cycle and post menstrual phase (P value < 0.01). However mid cycle values of IgA were not significantly lower than post menstrual levels ($P > 0.1$).

Numerically values of IgG were highest during premenstrual phase and lowest during mid cycle.

IgG/IgA ratios were lowest during mid cycle (1.6) IgG/IgA ratio was highest during post menstrual phase.

Subject number 45 did not show any immunoglobulin in cervical mucus. Subject number 42 and 43 did not have any IgA in their cervical mucus.

Mean value of IgA ± SD (mg/dl) for normal women serving as control (n=21) was 107.2±59.4 and of IgA ± SD (mg/dl) was 49.0±35.3. IgG/IgA ratio of the mean was 2.2

Table - II(b)

Percent increase in immunoglobulin concentration in various phase of M.C.

Ig type	Phase of cycle	Present increase (%)
IgG	Premenstrual vs postmenstrual	61.3
IgA	Premenstrual vs postmenstrual	108.4

$$\text{Present increase} = \frac{\text{Mean Ig conct. (postmenstrual)} - \text{Mean Ig conct. (postmenstrual)}}{\text{Mean Ig conct. (postmenstrual)}} \times 100$$

Table II (b) reveals that percent increase in IgA was more striking (108.4%) than IgG (61.3%) when mean Ig concentration in cervical mucus were compared during pre and post menstrual phase.

Immunoglobulin levels in cervical mucus of women during trimesters of pregnancy

As already noted 7 women were selected from first trimester, 7 from second trimester and 6 from third trimester of pregnancy. Results are shown in the table-III.

Mean values of IgG (± S.D.) during first second and third trimesters of pregnancy were 89.0 mg/dl (± 58.9)

114.9 mg/dl (±26.4), and 119.5 (±71.6) mg/dl respectively. Mean IgA values ± S.D. (mg/dl) were 31.1±18.6, 43.4±15.7 and 53.4±32.9 for first second and third trimesters respectively.

It was observed (table-III) that mean value of both IgG and IgA both increased gradually over 3 trimesters of pregnancy. Lowest values were observed in first trimester and highest values were obtained in third trimesters of pregnancy. But these results were not statistically significant (P value $\nearrow$ 0.05). In other words Ig values in first trimester of pregnancy were not significantly lower than that in second and third trimesters of pregnancy. Similarly, there was no statistically significant difference of Ig levels in second and third trimesters of pregnancy.

Mean IgG/IgA ratios in first second and third trimesters of pregnancy were 2.9, 2.6, 2.2 respectively. Thus there was a decreasing trend in the ratio within progress of pregnancy. Thus there was relatively more increase in IgA levels as compared to IgG levels with the progress of pregnancy.

Mean levels of IgG and IgA ± S.D. (mg/dl) in the whole group (b) were 107.5±2+48.1 and 42.0+23.5 respectively.

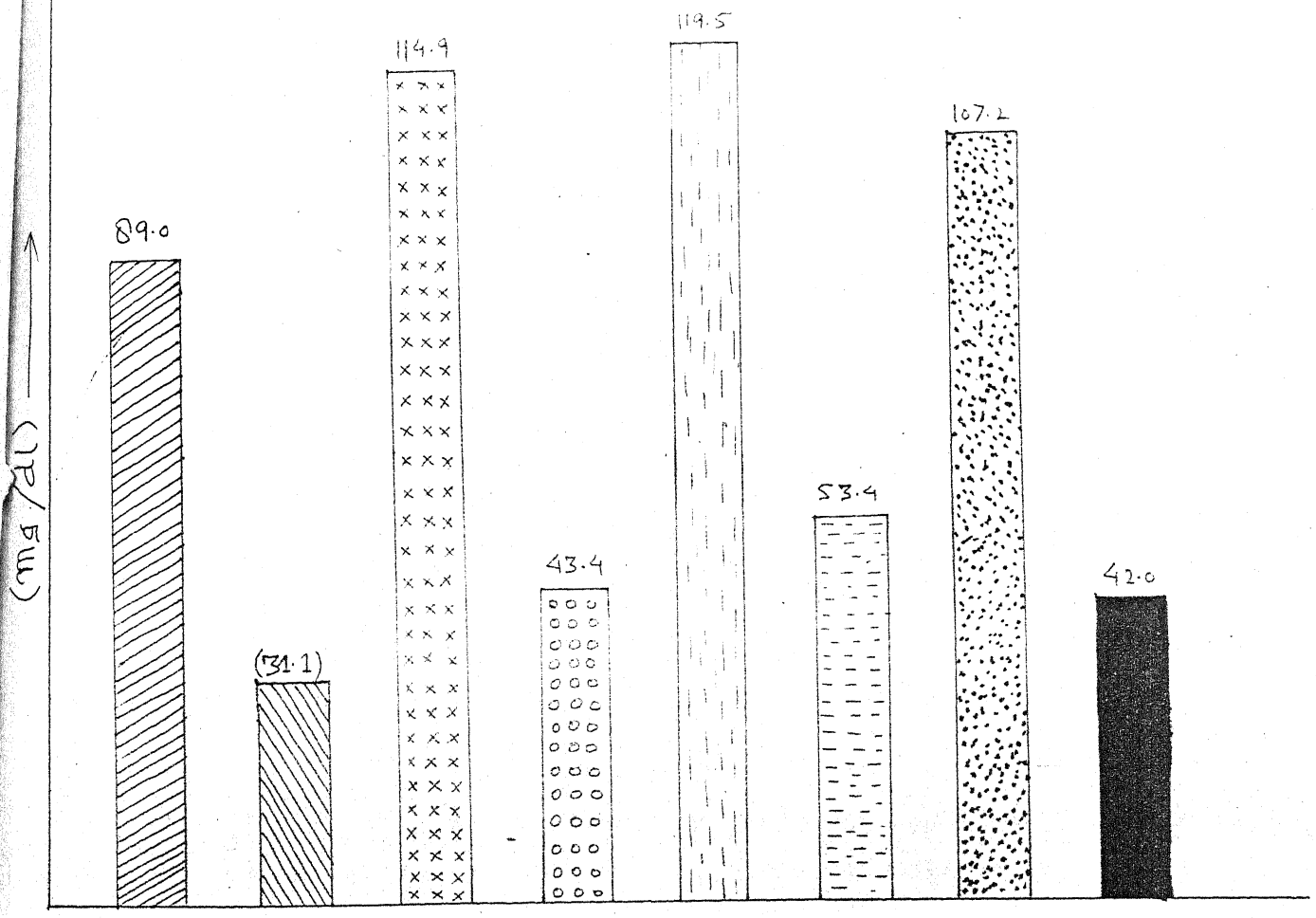
Mean IgG/IgA ratio in pregnancy was 2.6.

Table - III(a)

Immunoglobulin levels in cervical mucus of pregnant women

Sl.No. of subject	Trimester of pregnancy	IgG (mg/dl)	IgA (mg/dl)	IgG/IgA ratio	Significance of difference
1	First	67.0	28.5	2.3	Group B1 vs B2 for IgG & IgA difference is insignificant $P > 0.05$
2	-do-	181.5	41.5	4.4	
3	-do-	0	0	0	
4	-do-	141.5	60.5	2.3	
5	-do-	84.0	27.0	3.1	
6	-do-	92.0	37.5	2.4	
7	-do-	57.0	23.0	2.5	
Group B1 (n=7) Mean ± S.D.		89±58.9	31.1±18.6	2.9	
8	Second	168.0	72.5	2.3	Group B2 vs B3. Both IgG and IgA levels are not significantly different ($P > 0.05$)
9	-do-	112.0	37.5	3.0	
10	-do-	96.0	47.0	2.0	
11	-do-	110.0	34.5	3.2	
12	-do-	115.0	53.5	2.1	
13	-do-	107.5	31.0	3.5	
14	-do-	85.0	27.5	3.1	
Group B2 (n=7) Mean ± S.D.		114.9±26.4	43.4±15.7	2.6	
15	Third	74.0	29.5	2.5	Group B1 vs B3. Difference between IgG and IgA levels are not significant. ($P > 0.05$)
16	-do-	131.0	51.0	2.6	
17	-do-	247.0	115.0	2.1	
18	-do-	138.5	60.0	2.3	
19	-do-	47.5	27.0	1.8	
20	-do-	79.0	37.5	2.1	
Group B3 (n=6) Mean ± S.D.		119.5±71.6	53.4±32.9	2.2	
Group B (B1+B2+B3) (n=20) Mean ± S.D.		107.2±48.1	42.0±23.5	2.6	

Diag (2) - Ig Levels during pregnancy (in cervical mucus)



IgG level in Ist T.M. (n=7)

IgA level in Ist T.M. (n=7)

IgG level in IInd T.M. (n=7)

IgA level in IInd T.M. (n=7)

IgG level in IIIrd T.M. (n=6)

IgA Level in IIIrd T.M (n=6)

Mean IgG level in pregnancy (n=21)

Mean IgA level in pregnancy (n=21)

Table - III(b)

Percent increase in Ig's with progress of pregnancy

Trimester	Increase in IgG (%)	Increase in IgA (%)
Second trimester vs first trimester	29.1	39.5
Third trimester vs second trimester	4.0	23.0
Third trimester vs first trimester	23.0	71.7

Immunoglobulin levels in cervical mucus of women with cervical pathology : (Table-IV)

This group consisted of two sub-groups viz C1 includings cases with proved cervical erosion (7) and endocervicitis (2) and C2 including cervical dysplasia cases (n=5). Thus total number of cases were 14.

Mean IgG levels $\pm$ S.D. (mg/dl) in cervical mucus of patients were 226.3$\pm$88.7 and 430.5$\pm$77.8 in group C1 and C2 respectively. These results showed a highly significant difference between two groups ($P < 0.01$). Levels in cases of cervical erosion/endocervicitis were quite low as compared to patients of cervical dysplasia.

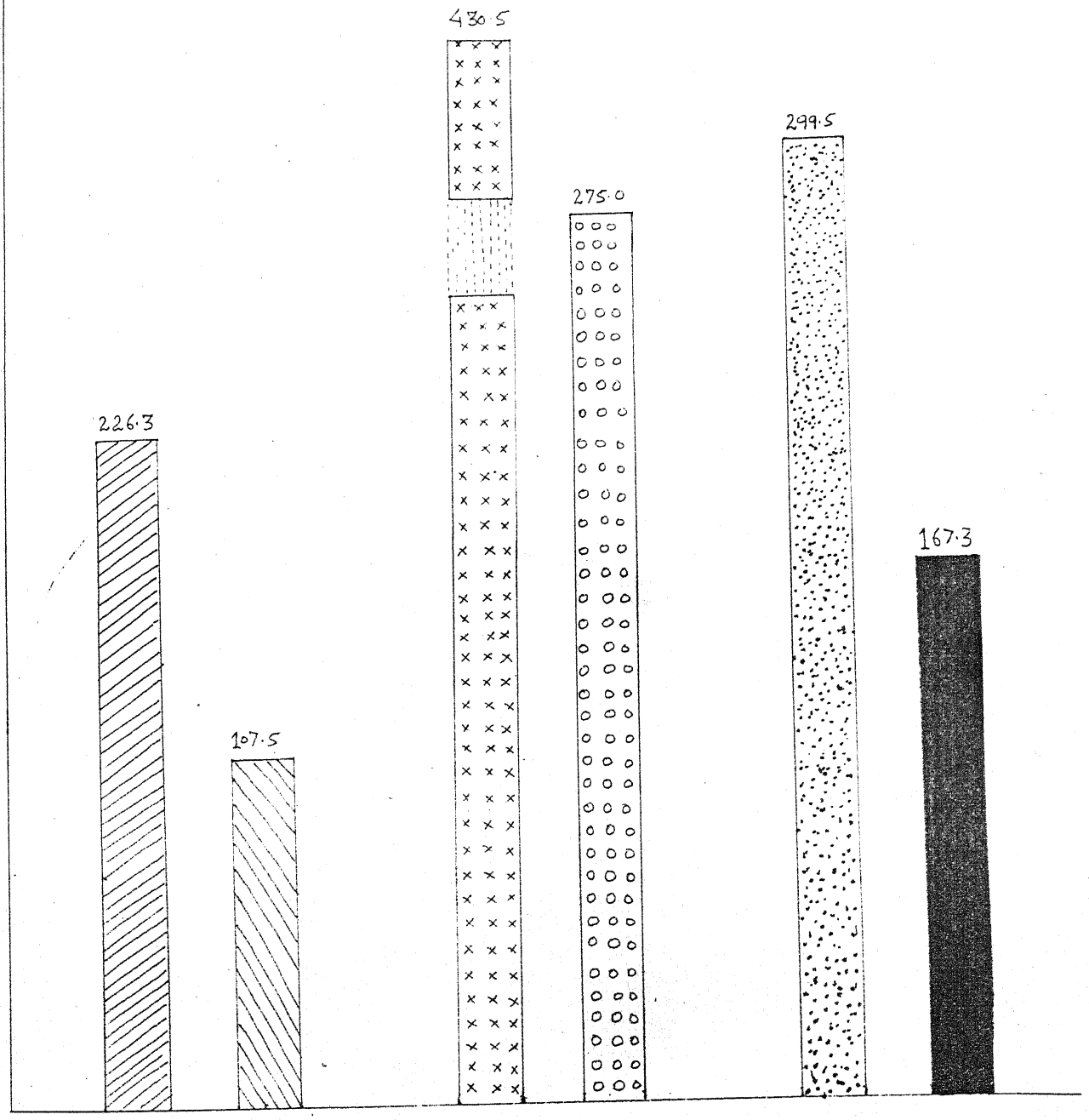
Mean IgA levels $\pm$ S.D. (mg/dl) were 107.5$\pm$34.3 and 275.0$\pm$61.4 in sub groups C1 and C2 respectively. Levels of IgA in cervical dysplasia (C2) group were significantly higher than those in cervical erosion/ endocervicitis group C1 (P value < 0.001).

Table - IV

Immunoglobulin levels in cervical mucus of women with cervical pathology

Sl.No. of subject	Diagnosis	IgG level (mg/dl)	IgA level (mg/dl)	IgG/IgA ratio	Significance of difference
21	Cervical erosion	231.5	137.5	1.7	IgG levels in group C1 was significantly lower than those in group C2 ($P < 0.01$)
22	- do -	188.5	94.0	2.0	
23	Endocervicitis	148.0	55.0	2.7	
24	- do -	378.0	1150.0	2.5	
25	Cervical erosion	253.5	114.0	2.2	
26	- do -	131.0	107.0	1.2	
27	- do -	290.0	141.5	2.0	
28	- do -	112.0	57.0	2.0	
29	- do -	304.5	111.5	2.7	
Group C1	Mean ± SD (n=9)	226.3±88.7	107.5±34.3	2.1	
30	Cervical dysplasia	422.0	223.5	1.9	IgA levels in group C1 were very significantly lower than those in C2 ($P < 0.001$)
31	- do -	405.5	238.0	1.7	
32	- do -	456.5	328.0	1.4	
33	- do -	327.5	231.0	1.4	
34	- do -	541.0	354.5	1.5	
Group C2	Mean ± SD (n=5)	430.5±77.8	275.0±61.4	1.6	
Group C (C1+C2)	(n=14) Mean±SD	299.2±124.5	167.3±93.9	1.8	

Diag (3) - Ig levels in diseased cervix (estimated in mucus)



IgG level in endocervicitis/cervical erosion (n = 9)

IgA level in endocervicitis/cervical erosion (n = 9)

IgG level in cervical dysplasia (n = 5)

IgA level in cervical dysplasia (n = 5)

Mean IgG level in diseased Cx. (n=14)

Mean IgA level in diseased Cx. (n = 14)

Mean IgG/IgA ratios were 2.1 and 1.6 respectively in C1 and C2 sub groups. Thus relative increase of IgA level as compared to IgG was found in cervical dysplasia group.

When these two sub groups were put together, mean IgG and IgA values ± S.D. (mg/dl) were 299.2±124.5 and 167.3±93.9 respectively. This gave an IgG/IgA ratio of 1.8.

Table - IV (b)

Percent increase Ig in cervical pathology group as compared to normal controls

Pathologic group	% increase in IgG	% increase in IgA
Endocervicitis/cervical erosion	111.1	119.2
Cervical dysplasia group	301.5	461.2

Immunoglobulin levels in normal controls compared with levels in pregnancy and cervical pathology (Table-V)

When IgG levels were compared it was found IgG levels in pregnancy were not significantly different from those during three phases of menstrual cycle (normal controls). P value was > 0.05.

Similarly IgA levels were not significantly different in these two groups.

IgG/IgA ratio in controls was lower (2.2) than in pregnancy 2.6.

IgG values of controls were significantly lower than those of patients of cervical erosion/endocervicitis or cervical dysplasea (P value < 0.01 and 0.001 respectively).

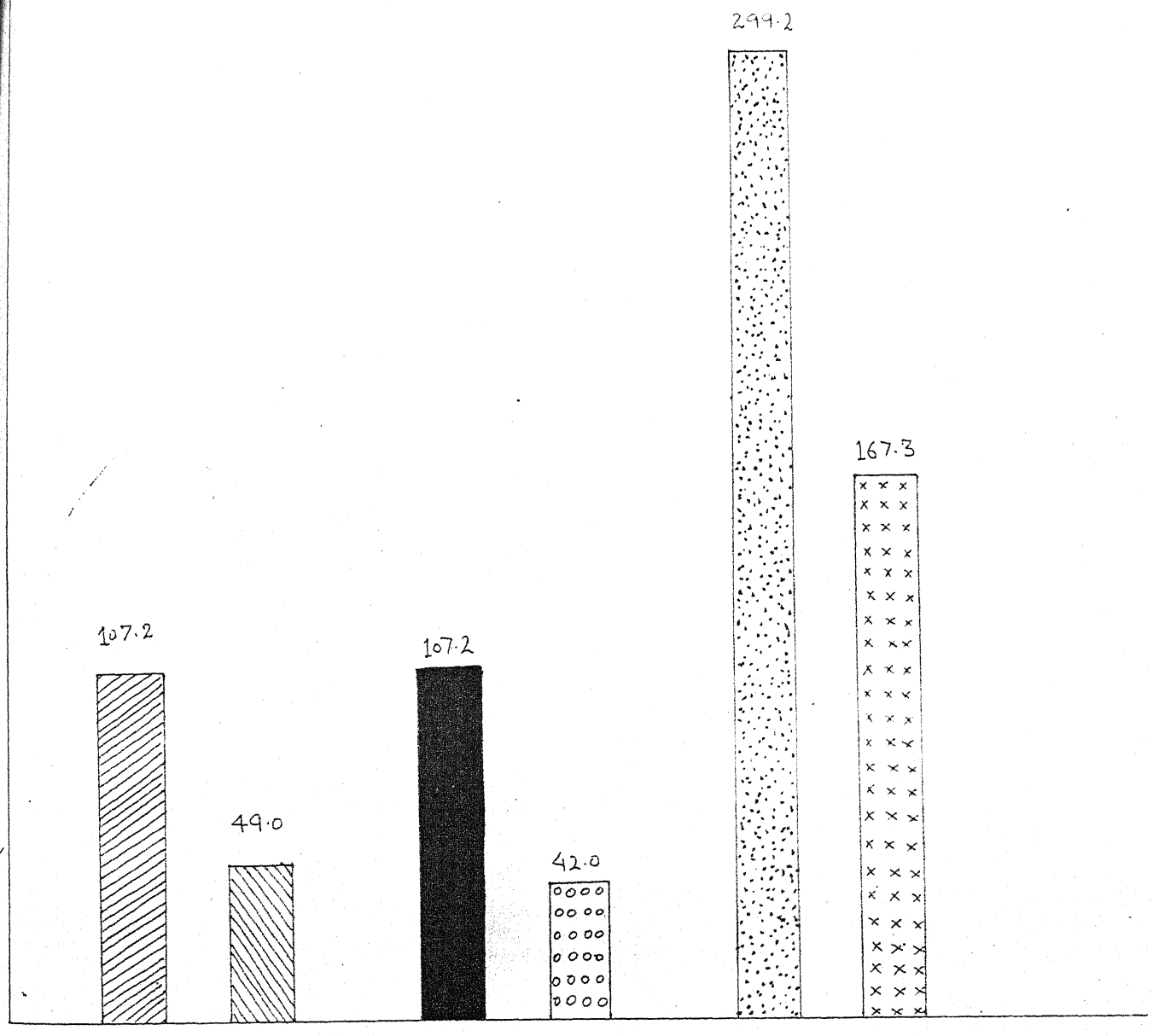
IgA values of controls were also significantly lower than those of cervical erosion/endocervicitis group or cervical dysplasia group (P < 0.001 and < 0.001 respectively).

Table - V

Comparative values of Ig's in cervical mucus in women in different groups

Sub group/ group	n	Mean IgG ± S.D. (mg/dl)	Mean IgA ± S.D. (mg/dl)	IgG/ IgA ratio	Significance of difference
Normal control (gp A)	21	107.2 ± 59.4	59.4 ± 35.3	2.2	IgG & IgA level in normal control vs pregnant women
Pregnant women (gp B)	20	107.2 ± 48.1	42.0 ± 23.5	2.6	Not significant (NS)
Cervical erosion/ endocervicitis sub group C1	9	226.5 ± 88.7	107.5 ± 34.3	2.1	IgG & IgA level in cervical erosion/endocervicis vs control: highly significant P< 0.01
Cervical dysplasia subgroup C2	5	430.5 ± 77.8	275.0 ± 61.4	1.6	IgG & IgA level in cervical dysplasia vs control highly significant P<0.001
Cervical pathology (gp C)	14	299.2 ± 124.5	167.3 ± 93.9	1.8	IgG & IgA level in cervical pathology vs control: highly significant P < 0.001

Diag(4) Mean Ig levels in different groups (in mucus of Cx)



IgG level in control g.p. (n=21)

IgA level in control g.p. (n=21)

IgG level in pregnancy (n=20)

IgA level in pregnancy (n=20)

IgG level in diseased Cx. (n=14)

IgA level in diseased Cx. (n=14)

IgG/IgA ratios were low in these two groups (2.1 in cervical erosion/endocervicitis group and 1.6 in cervical dysplasia group), than controls (2.2). However difference was significant only in cervical dysplasia group where IgA was relatively more increased.

Levels of Ig's in cervical pathology group as a whole were higher as compared to controls and results were very highly significant ($P < 0.001$).

DISCUSSION

DISCUSSION

The present study is an attempt to establish the average immunoglobulin concentrations in cervical mucus of normal women during various phase of menstrual cycle, pregnant women and women with various cervical pathologies including endocervicitis cervical erosion and cervical dysplasia in cases which were histologically proved.

1. Age of patients

The average age of control group was 21.2 years (ranged 18 to 25 years). The pregnant group ranged from 18-29 years (average 21.9 years). The average age of patients with cervical pathology was 25.1 years (range 20-33 years). Thus the average age of control and pregnant group was almost identical but those in cervical pathology group were a little older. Weldmen et al (1971) have reported decrease in percentage of IgA and increase in that of IgG, in cervicovaginal secretions with increasing age. Present study didnot shown any correlation of these levels with age. In another study of influence of aging on external secretions, Alford (1968) found a decrease in nasal wash.

IgA concentrations with increasing age.

2. Nature of antibodies in cervical mucus

The human endocervix is lined with secretory columner epithelium which produces mucus shown to contain immunogl-

obulins (Moghissi and Newhous, 1962; Schumacher et al 1965; Herve et al 1965, Elstein and Pollard, 1968). Specific antibodies in cervical mucus have been demonstrated against spermatozoa (Solish et al 1961; Strauss, 1965), blood group antigens, E. Coli and C. albicans (Parish et al, 1967). Local formation of antibody is suggested by finding of higher titres in mucus than in serum (Parish et al, 1967), the detection of secretory piece and lactoferrin in endocervical epithelium by immunofluorescence (Masson et al, 1968; Hulka and Omran, 1969; Tourville et al 1970), and the location of IgA and IgG containing plasma cells in the underlying stœoma by the same method (Masson and Ferrin, 1969). Moreover, Hutcheson et al (1974) have incriminated local antibody in the aetiology of infertility, they demonstrated increased numbers of IgA + containing plasma cells in cervical biopsies from patients with unexplained infertility.

In the female reproductive tract local antibody production was first investigated in heifers by Kerr and Robertson (1941) who showed that vaginal secretions contained agglutnins to trichomones foetus when locally infected by these organisms. This has been subsequently confirmed in heifers (Kerr and Robertson 1943), in

cattle (Hughes 1953; Kerr and Robertson 1953; Kan 1955) cows (Pierce 1946). Pierce (1946) also studied Ig in serum besides mucus and observed higher titres in cervical mucus of cases infected by trichomonas foetus than serum. Batty and Warrack (1955) and Bell and Wolf (1967) found similar results in rabbit and straus (1961) in humans.

In the present study, simultaneous studies of IgG and IgA levels were not done in serum. Yet the results obtained from cervical mucus showed that there was local antibody production. Cervical mucus IgG/IgA ratio of 2.2 in normal controls 2.6 in pregnant women and 1.8 in women with cervical pathology is quite different from that in serum 5:1 to 8:1 Coughlan and Skinner (1977) also obtained an IgG/IgA ratio of 2:4. Similarly Behrman and Lieberman reported a ratio of 2:3. Thus present findings are similar to those of above two studies. Findings of Firemans et al (1963) are also in agreement with the findings of this study. However, these findings are contrary to those of Chodirker and Tomasi (1963), Tomasi and Bienestock (1963) and 1968) and Masson Ferrin (1969) who reported relatively high concentration of IgG.

Since IgG/IgA ratio is significantly lower than that found in serum this provides indirect evidence of local antibody production in cervical mucus.

While there is a good evidence that IgA is produced locally in the cervix (Wald man et al 1972; Ogra and Ogra, 1973, Rebells et al 1975), Coughland and Skinner (1977) are not clear about the origin of IgG in cervical mucus. Von Kaulla et al (1957) believe that mucus IgG originates by passive transudation in serum. Coughlan and Skinner (1977) reported no positive correlation between serum and mucus IgG concentrations. Basing on positive correlation between cervical mucus IgG and IgA concentration they argued that there was a possibility that influences controlling IgA production may also be involved in part at least in local IgG production.

Amino et al (1978) observed gradual decreases in serum concentration of IgG, IgA and IgM during normal pregnancy. In the present study cervical mucus no such decrease in IgA and IgG levels with progress of pregnancy (Table-III) was observed suggesting an independent production of IgG and IgA into cervical mucus for reproductive tract.

In the present study the levels of IgG and IgA were very high in cervical mucus of patients with cervical pathology (mean IgA 167.3±93.9; mean IgG 299.2±124.5) as compared to normal controls (mean IgA 49.0±35.3; mean IgG 107.2±59.4). These observations might suggest a

selective loss of selective serum/mucus transudation in abnormal cervices (Coughlan and Skinner, 1977). Schumacher et al (1965) also suggested similar mechanism. But this does not explain higher level of IgA (IgG/IgA ratio of 1.8) in diseased cervix as compared to that in normal controls (IgG/IgA ratio 2.2). This observation suggests the possibility of local response to abnormal epithelial cells or to a prolonged antigenic stimulus. Bhatia et al (1981) conclusively showed that increased production of IgG and IgA at local level in such conditions when they could not find a simultaneous local increase in complement (C_3 & C_4), albumin and transferrin.

To summarize, present study suggests a local production of IgA and IgG into cervical mucus and not merely a transudation of serum proteins, a review held by previous workers like Von Koulla (1957), Tomasi and Bienestock (1963 & 1968) and Masson and Ferrin (1969).

3. Immunoglobulin levels in normal controls (during various phases of menstrual cycle :

As shown in table II mean values of IgG ± SD (mg/dl) in premenstrual, mid cycle and post menstrual phases were 171±46.9, 44.3±34.3 and 106.3±15.3 respectively. On the other hand, mean IgA values ± SD (mg/dl) were 81.1±2.8, 27.3±27.5 and 38.9±17.6 in premenstrual mid cycle and postmenstrual phases, respectively. Thus the average IgG value ± SD (mg/dl) in normal controls

(n=21) was 107.2±59.4 while IgA value ± SD (mg/dl) was 49.0±35.3.

It is evident from above data that Ig levels were lowest during mid cycle phase and highest during premenstrual phase. These findings are in agreement with Elstein and Pollard (1968) who found detectable immunoglobulin in pregestational phase only. Tourville et al (1970) also reported increase in immunoglobulin IgG and IgA in secretory phase of menstrual cycle. Elstein (1970) also reported similar increase in immunoglobulin level in premenstrual phase.

Schumacher (1973), Davis et al (1983), Bhatia et al (1981) who measured IgG and IgA in mucus also found mid cycle nadir of these Ig's and peak in their levels in premenstrual phase. Coughlan and Skinner (1977) failed to demonstrate mid cycle decrease but did shown increase of IgG and IgA in premenstrual phase.

Harve et al (1965) on the contrary reported a mid cycle peak of immunoglobulin which they attributed to follicular fluid as it did not occur in anovulatory cycle. The present study included only fertile women with regular menstrual cycles. It is unlikely that most if not all women included in this study had anovulory cycles. However, it may be pointed out that patient

No. 45 did not have any detectable immunoglobulin of either class at mid cycle. But this may be due to some technical error in dilution/storage of cervical mucus rather than anovulatory cycle.

These findings of present study do not confirm to observations of a number of workers viz Schumacher (1965) Solish et al (1961), Moghissi and Neuhaus (1966), Waldman et al (1971), Chipperfield and Evans (1972) and Eissa et al (1985) who could not demonstrate any variation in immunoglobulin levels of either class with the phases of menstrual cycle.

It is evident from above studies that there is a lot of controversy regarding changes in Ig levels with various phases of menstrual cycle. It may be due to difference between method of collection or storage of cervical mucus or technique employed for estimation of immunoglobulin levels. But in present study the increase in IgG as well as IgA levels in premenstrual phase was highly significant ($P < 0.01$) when levels were compared with those in post menstrual phase.

Though the predominant immunoglobulin was IgG yet the increase in premenstrual phase was more evident in IgA (108.3%) as against IgG (61.3%). Similar findings were observed by Bhatia et al (1981) who showed relatively more increase in IgA level in premenstrual phase

than IgG though the predominant Ig was still IgG. Coughlan and Skinner (1977) also found relatively greater increase in IgA. But Schumecher (1973) found relatively greater increase in IgG. Present findings are in contrary to Waldman et al (1971) and Chipperfield and Evans (1972) who reported IgA as predominant immunoglobulin of cervical mucus.

Study	Mean IgG/ range in normal control	Mean IgA/ range in normal control
Waldman et al (1971)	12 mg/dl	22 mg/dl
Hulka & Omran (1969)	9-456 mg/dl	2.8-90 mg/dl
Eissa et al (1985)	420±7 mg/dl	21±4 mg/dl
Coughlan and Skinner (1977)	131.8±11.7 mg/dl	47.1±7.2
Masson et al (1969)	45 mg/dl	9 mg/dl
Bhatia et al (1985)	Mean 60.2 mg/dl	Mean 32.9/dl

If we have a look over table No. VI, than it is evident that levels found in present study are quire similar to those of Coughlan and Skinner (1977), Hulka and Omran (1969). But levels quoted by Eissa et al (1985) Masson et al (1969) and Waldman et al (1971) were quite low.

In only other study from India (Bhatia et al) IgG and IgA values were almost half to 2/3 that of present

study. But that study used immunoelectrophoratic method (Holbrow and Johnson technique) for estimation of Ig unlike present study using radial immunodiffusion. Moreover there was difference in method of collection, dulutation and storage of cervical mucus. These differences might explain this dissimilarity between two different Indian studies. Similar differences also existed in western studies.

Coughlan and Skinner (1977) did not find any difference in IgG/IgA ratio during various phases of menstrual cycle. Bhatia et al (1981) also did not show any significant change in ratio during various phases. But present study, like Hulka and Omran (1969) found a relative decrease in IgG/IgA ratio (1.6 midcycle as against 2.2 and 2.1 in post and premenstrual phase). Hulka and Omran (1971) found a mid cycle decrease reaching nadir at the day of ovulation. Present study though did not make a record of day of ovulation yet it confirms to the findings of these authors.

4. Immunoglobulin levels in cervical mucus during pregnancy

As shown in table (III) mean values of IgG±S.D. (mg/dl) observed in first second and third trimesters of pregnancy were 89±58.9, 114.9±26.4 and 119.5±71.6 respectively; while respective values for IgA were

31.1±18.6, 43.4±15.7 and 53.4±32.9. Thus there was a gradual increase in immunoglobulin levels with the progress of pregnancy. But this increase was not statistically significant when results were compared using student 't' test.

The percent increase in IgA was more as compared to IgG, with progress of pregnancy. IgA showed a 71.7% increase while IgG only 23% from first trimester to third trimester. In the present study IgG was the dominant immunoglobulin which is in agreement with Bhatia et al (1981). In both the studies increase in IgA was more prominent as compared to IgG.

Relatively higher levels of immunoglobulins were detected in present study as compared to those reported by Bhatia et al (1981).

Present observations are not in agreement with those of Waldman et al (1971) who showed no change in immunoglobulin levels with the progress of pregnancy. Levels observed in their studies were as such quite low (IgG 9 to 11 mg% and IgA 1.3-25 mg/dl). Predominant immunoglobulin in their study was IgA not IgG. These results might be so due to difference in technique of collection and storage of cervical mucus.

Amino et al (1978) reported a decrease in serum immunoglobulin levels with the progress of pregnancy

which they attributed to mainly immunosuppression. Present study over cervical mucus shows opposite trend. This observation suggests production of local antibodies by human female genital tract which is governed by an independent mechanism.

Marculis et al (1971) observed that concentration of serum IgG diminished with each successive trimester whereas concentration of other two immunoglobulin IgM and IgA were either unchanged or had no constant trend. Similar were the observations of Tiselius (1937).

Brown et al (1954), Gudson (1989) and Gangun et al (198) who besides a fall in IgG levels also observed a fall in IgM levels, with no change occuring in IgA levels.

As far as this present study is concerned we have found a progressive increase in immunoglobulin levels of IgG and IgA, though the dominant immunoglobulin is IgG the percentage increase of IgA was 71.7% as compared to IgG which was only 23%. Hence we can now say according to the present study the cervix play a role in the production of local antibodies, independent of the levels of serum.

5. Immunoglobulin levels in women with cervical pathology

As evident from table V and VI both IgG and IgA was were quite high in cervical pathology group as compared to

normal controls. Results were significant for cervical erosin/endocervicitis group. But they were more significant in cervical dysplasia group. Corresponding percent increased in IgG and IgA reflected the same trend. In cervical erosion/endocervicitis group percent increase in IgG was 111.1 and in IgA was 119.2%. In cervical dysplasia group it was 301.5% for IgG and 461.2% for IgA. Schumacher et al (1965) have also reported increase in immunoglobulin level in cervical mucus of patients with non malignant abnormal cytology. Like present study Bhatia et al (1981) and Coughlan Shimmer (1977) noted quite significant increase in IgG and IgA in patients with abnormal cytology. Bhatia et al (1981) who differentiated between cervical pathologies found more increase in patients with malignant group as compared to non malignant group. They also noted a predominant increase of IgA. On the other hand Waldman et al (1971) found no significant difference with cervicitis.

According to Schumacher et al (1965) this relative increase in IgG and IgA may be due to loss of selective serum/mucus transudation in abnormal cervices, in patients with abnormal cytology. But this does not, however, explain relatively greater increase in

IgA. Concentrations in patients with abnormal cytology, and it is possible that increased immunoglobulin concentrations may represent a local response to abnormal epithelial cells or to a prolonged antigenic stimulus from an exogenous or endogenous infections agent (Coughlan and Skinner, 1977). To refute the theory of loss of selective barrier between serum and mucus as profounded by Schummacher et al (1965). Bhatia et al (1981) made a simultaneous study of cervical mucus C_3 and C_4 albumin and transferrin levels in patients with cervical pathology. These levels were not suggestive of loss of any such barrier strengthening the suggestion of Coughlan and Skinner (1977) that this might be due to prolonged antegenic stimulation by endogenous or exogenous agent. In the view of Coughlan and Skinner (1977) type 2 herpes simplex virus a known associate of cervical carcinoma (Skinner et al 1971, Skinner 1976) and an agent capable of establishing latent infection in the human subject (Sterens et al 1972 and Baringer, 1974). Would seem a reasonable candidate

Relatively more increase of IgA in presentstudy with female genital tract infection (endocervicitis) is also supported by previous studies (Chipperfield and Evans 1972; Waldman et al 1972; Ogra and Ogra 1973 etc.).

Chipperfield and Evan (1972) however, did not find any relation between cervical erosion and immunoglobulin level.

Present study reemphasizes the suggestion of Bhatia et al (1981) that estimation of immunoglobulin level particularly of IgA and IgG should be done as an early diagnostic aid for cervical malignancies. It is agreed that investigation of cervical mucus for neutralizing antibody or any other antibody the type two herpes simplex virus as suggested by Coughlan and Skinner (1977). But in places where such advance facilities are not available this simple estimation of mucus IgA and IgG besides cytological examination might prove quite helpful in early diagnosis of cervical dysplasia and other premalignant or malignant conditions.

CONCLUSION

SUMMARY AND CONCLUSIONS

The present study was conducted in Department of Obstetrics and Gynaecology, M.L.B. Medical College, Jhansi.

Total 55 cases were included in the study out of these 21 were normal control women in three different phases of menstrual cycle i.e. 7 each from post menstrual, Pre menstrual and mid cycle phase. Women in the different trimester of pregnancy 7 each from Ist and IInd trimester and 6 from the IIIrd trimester. The third group comprised up of women having cervical pathology like cervical erosion endocervicitis and dysplasias (in their different grades).

Cervical mucus was collected by cervix directly with the help of tubuculin syringe without needle and diluted with known volume of saline and stored at -20°C. Estimation of immunoglobulin levels (IgG/IgA) was done by radial immunodiffusion.

The results obtained were :

- Mean level	IgG	IgA
I- Menstrual cycle (Normal control)	107.2±59.4	49.0±35.3
II- Pregnant women	107.20±48.15	42.0±23.53
III - Cervical pathology	299.21±124.52	167.32±93.90
a) Endocervicitis/ cervical erosion	226.27±88.66	107.5±34.29
b) Dysplasia	430.5±77.81	275.0±61.41

Following conclusions were drawn from this study :

1. That age does not exert any effect on the level of IgA or IgG in cervical mucus contrary to that observed by Waldman et al (1971).

2. Human female genital tract is a local antibody secretor. This is reflected by IgG/IgA ratio 2.2 in normal controls, 2.6 in pregnant women and 1.8 in women with cervical pathology. In serum this ratio ranges from 5 to 8. It suggests that IgG found in mucus are not derived from blood. Moreover, with the progress of pregnancy a relative increase in immunoglobulin levels was noticed which is in contrast to serum levels in pregnancy (Ammeni et al 1978 and many other workers). Where a decrease occur with progress of pregnancy.

3. Immunoglobulin levels are minimum at mid cycle and maximum during premenstrual phase. The decrease in Ig levels at mid cycle is quite significant which can not be explained on the basis of dilution of mucus alone (which is normal at mid cycle). Some other cause should be sought by further studies.

4. More studies are required to prove the present observations. There is lot of controversy regarding change in Ig levels with menstrual cycle. Methodology of cervical mucus collection and storage should be standardized.

5. The premenstrual increase in Ig may be reflecting a protective mechanism of human uterus/cervix to protect early conceptus from infection (a review just suggested by Coughlan and Skinner 1977).

6. There is no statistically significant difference between pregnancy and normal controls as far as mean levels of IgA and IgG are concerned. But in pregnancy a rising trend in IgG particularly IgA was observed with the pregnancy.

7. Very high levels of IgA and IgG were observed in patients with cervical pathology particularly those with cervical dysplasia. It is suggested that estimation of IgG and IgA should be done in patients with suspected cervical malignancies as diagnostic aid.

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MASTER TABLE

Sl. No.	Selection of cases	Age in years	Obsterical history	Menstrual history (cycles)	P/A Exam. (FH)	PSExam. Exam.	P/V Exam. (Uterus) NS/AV*	Socio-economic Status LM/L/U@	IgG mg/dl	IgA mg/dl	G/A ratio
1	2	3	4	5	6	7	8	9	10	11	12
1	Ist trimester	22	$G_3P_2A_0$	Previous cycles regular	-	-	8 wks. AV soft	LM	67.0	28.5	2.35
2	- do -	20	$G_2P_1A_0$	- do -	-	-	12 wks soft	L	181.5	41.5	4.37
3	- do -	24	$G_4P_2A_1$	- do -	-	-	6 wks AV soft	L	0	0	0
4	- do -	19	$G_1P_0A_0$	- do -	-	-	8 wks AV soft	U	141.5	60.5	2.35
5	- do -	22	$G_5P_3A_1$	- do -	-	-	12 wks soft	LM	84.0	27.0	3.1
6	- do -	23	$G_4P_3A_0$	- do -	-	-	6 wks AV soft	L	92.0	37.5	2.45
7	- do -	24	$G_5P_4A_0$	- do -	-	-	6 wks AV soft	L	57.0	23.0	2.47
								Mean ± S.D.	89±58.84	31.1±18.57	2.86
8	IInd trimester	22	$G_2P_1A_0$	Previous cycles regular	F/H-22 wks VX Ballotment+	-	-	LM	168.0	72.5	2.31
9	- do -	25	$G_4P_1A_2$	- do -	F/H-24 wks Ballotment+	-	-	L	112.0	37.5	2.98
10	- do -	18	$G_1P_0A_0$	- do -	18 weeks Ballotment+	-	-	LM	96.0	47.0	2.04
11	- do -	20	$G_2P_1A_0$	- do -	20 weeks Ballotment+	-	-	M	111.0	34.5	3.21
12	- do -	25	$G_1P_0A_0$	- do -	24 weeks Ballotment+	-	-	LM	115.0	53.5	2.13
13	- do -	20	$G_3P_1A_1$	- do -	18 weeks Ballotment+	-	-	LM	107.5	31.0	3.46
14	- do -	18	$G_4P_2A_1$	- do -	20 weeks Ballotment+	-	-	L	85.0	27.5	3.09
								Mean ± S.D.	114.85± 26.41	43.35± 15.74	2.6
15	IIIrd trimester	22	$G_6P_4A_1$	Previous cycles regular	32 weeks FHS + R	-	-	L	74.0	29.5	2.50
16	- do -	20	$G_3P_2A_0$	- do -	28 weeks FHS + R	-	-	LM	131.0	51.0	2.56
17	- do -	22	$G_4P_2A_1$	- do -	34 weeks FHS + R	-	-	L	247.0	115.5	2.31

1	2	3	4	5	6	7	8	9	10	11	12
18	IIIrd trimester	29	$G_3P_2A_0$	Previous cycles regular	28 weeks FHS + R	-	-	LM	138.5	60.0	2.30
19	- do -	23	$G_2P_1A_0$	- do -	32 weeks FHS + R	-	-	M	47.5	27.0	1.75
20	- do -	20	$G_3P_1A_1$	- do -	30 weeks FHS + R	-	-	L	79.0	37.5	2.10
							Mean ± S.D.		119.5± 71.6	53.41± 32.94	2.23
21	Cervical erosion	22	P_{4+0}	4/28 flow adequate	-	Erosion on anterior lip	UT-NS AV	L	231.5	137.5	1.68
22	- do -	21	P_{6+0}	3/28 regular spotting off and on	-	Cervical erosion on both lips. Bleeds on temp.	UT-RV NS	LM	188.5	94.0	2.0
23	Endo cervicitis	22	P_{4+0}	4/22 flow adequate	-	Cervix hypertroptised discharge+	UT MP size RU	L	148.0	55.0	2.89
24	Cervical erosion	20	P_{3+0}	4/28 flow adequate	-	Cervical erosion anterior lip	UT-RV NS	LM	378.0	150.0	2.52
25	Endocervicitis	26	P_{6+1}	3/26 flow adequate	-	Antr. lip cervix hypertroptised discharge+	UT-AV NS	L	253.5	114.0	2.22
26	Endocervicitis	24	P_{4+2}	4/28 flow adequate	-	Cervical discharge+	UT-RV NS	LM	131.0	107.0	1.22
27	Cervical erosion	23	P_{5+0}	6/28 flow	-	Erosion on both lips	UT-AV NS	L	290.0	141.5	2.04
28	Cervical erosion	25	P_{6+0}	3/20 flow adequate	-	Erosion on postr. lip	UT-RV NS	LM	112.0	57.0	1.96
29	Cervical erosion	22	P_{3+2}	4/28 flow adequate	-	Erosion on postr. lip	UT-RV NS	LM	304.0	111.0	2.70
							Mean ± S.D.		226.27± 88.66	107.5± 34.29	2.11
30	Cervical dysplasia Grade-I	28	P_{3+0}	6/28 flow adequate	-	Cervix hypertrophied	UT-RV NS	L	422.0	223.5	1.9
31	Cervical dysplasia Grade-II	29	P_{4+0}	4/28 flow adequate	-	Cervical discharge + cervix hypertrophied	UT-RV NS	LM	405.5	238.0	1.7
32	Cervical dysplasia Grade-II	30	P_{6+0}	Irregular flow Adeq.	-	Cervix hypertrophied discharge+	UT-AV NS	L	456.5	328.0	1.41
33	Cervical dysplasia Grade-III	28	P_{3+0}	4/26 flow adequate	-	Cervix hypertrophied	UT-AV NS	M	327.5	231.0	1.42
34	Cervical dysplasia Grade-III	33	P_{5+0}	2/26 flow adequate	-	Cervix hypertrophied	UT-RV NS	L	541.0	354.5	1.53
							Mean ± S.D.		430.5± 77.81	275.0± 61.41	1.6

(Continued....)

1	2	3	4	5	6	7	8	9	10	11	12
35	Pre menstrual	20	P_{4+0}	3/26 regular	-	NAD	UT-RV NS	L	112.0	41.5	2.7
36	- do -	18	P_{3+1}	4/28 regular flow adequate	-	,,	UT-RV NS	U	237.5	123.5	1.9
37	- do -	25	P_{5+0}	4/28 regular flow adequate	-	,,	UT-AV NS	LM	207.0	78.5	2.6
38	- do -	20	P_{2+1}	4/26 regular	-	,,	UT-AV NS	L	171.0	57.5	2.03
39	- do -	22	P_{2+1}	21/28 regular	-	,,	UT-RV NS	L	127.0	57.5	2.10
40	- do -	23	P_{4+0}	3/26 regular	-	,,	UT-RV NS	L	147.5	75.0	1.96
41	- do -	20	P_{2+1}	4/28	-	,,	UT-RV NS	L	201.5	108.0	1.86
								Mean ± S.D.	171± 46.87	81.48± 27.98	2.11
42	Mid cycle	22	P_{6+1}	4/28 flow adequate	-	NAD	UT-RV NS	L	31.5	0	31.5
43	- do -	20	P_{4+2}	4/28 flow adequate	-	,,	UT-AV NS	LM	27.5	0	2.7
44	- do -	23	P_{3+0}	3/26 flow adequate	-	,,	UT-RV NS	M	67.5	59.0	1.2
45	- do -	22	P_{2+0}	52/26 flow adequate	-	,,	UT-AV NS	L	0	0	0
46	- do -	18	P_{1+0}	4/30 flow adequate	-	,,	UT-AV NS	LM	84	56.5	1.4
47	- do -	20	P_{2+0}	3/30 flow adequate	-	,,	UT-AV NS	LM	78.0	48.5	1.6
48	- do -	24	P_{3+0}	4/30 flow adequate	-	,,	UT-AV NS	L	82.0	27.0	2.6
								Mean ± S.D.	44.5± 34.29	27.28± 27.51	1.63
49	Post menstrual	22	P_{5+0}	4/30 flow adequate	-	NAD	UT-RV NS	LM	89.0	34.5	2.57
50	- do -	23	P_{4+0}	3/26 flow adequate	-	,,	UT-AV NS	LM	108.5	48.0	2.26
51	- do -	24	P_{2+3}	4/26 flow adequate	-	,,	UT-RV NS	LM	118.5	66.0	1.79
52	- do -	22	P_{4+0}	3/26 flow adequate	-	,,	UT-RV NS	L	88.0	15.0	5.86
53	- do -	18	P_{3+0}	4/26 flow adequate	-	,,	UT-AV NS	L	94.0	37.5	2.50
54	- do -	20	P_{4+0}	6/30 flow adequate	-	,,	UT-AV NS	LM	113.5	21.0	5.40
55	- do -	19	P_{2+0}	5/30 flow adequate	-	,,	UT-AV	LM	131.0	50.0	2.62
								Mean ± S.D.	106± 15.28	38.85± 17.57	2.72

*NS = Normal Size
AV = Ante verted

@L = Lower
LM = Lower Middle
U = Upper